

INVESTIGATIONS CONCERNING THE REPRODUCTIVE BEHAVIOUR OF *MOLLIENISIA* "*FORMOSA*"¹

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(With Plate X and Thirteen Text-figures)

AMONG the species of *Mollienisia*, a genus of poeciliid cyprinodonts, hybridization occurs and often results in the production of hybrids of peculiar reproductive habits and conditions.

Over a large part of the range of *Mollienisia*, there is a form of hybrid origin which Dr Carl L. Hubbs calls *M. "formosa"* (Pl. X, fig. 1). In appearance it is intermediate between *M. sphenops* (Pl. X, fig. 2) and *M. latipinna* (Pl. X, fig. 3). This form exists only as females. Out of two thousand specimens examined from Tamaulipas and Texas, Dr Hubbs has not found a single male. This raises the question as to how such a form can continue its existence. In the Tamaulipas region, where it lives well inward of the coastwise range of *M. latipinna*, it is found with *M. sphenops* and supposedly mates with males of that group. In Texas, where it abounds in the recesses of the Brownsville region, considerably farther north than the *M. sphenops* occurs, it is found with *M. latipinna* and here presumably mates with males of this group. These presumptions have been verified by means of extensive aquaria tests performed by C. L. & L. C. Hubbs (1932). Several of these forms, *M. "formosa"*, from Forlorn in Tamaulipas, which had presumably mated with males of *M. sphenops*, and others from Brownsville, Texas, which had presumably mated with the very different males of *M. latipinna*, have produced young in the laboratory after having been transported from their natural habitats. Not one of the young produced from these females have in their adult condition shown any approach toward the characters of the male involved. The characters of the mother seem to have been inherited as a whole. Although the broods have been large and numerous, not a single male has appeared among them. These results and presumptions have been verified also by control matings. Virgin females of this unisexual species mate readily with males of either parent species and soon become pregnant, but the result is always the same, female offspring with

¹ Contribution from the Department of Zoology, University of Michigan.

330 *The Reproductive Behaviour of Mollienisia "formosa"*

all the characters of their mothers. This has been demonstrated consistently in stocks from both Tamaulipas and Texas. These localities are shown on the map (Text-fig. 1).

Given an explanation as to how this group continues its existence, two other questions become of importance, namely, what type of reproduction will account for the production of purely female broods of young and what is the cause of the purely matroclinous inheritance? In the paper by C. L. & L. C. Hubbs the results of their investigations on



Text-fig. 1. Map showing the approximate distribution of *Mollienisia*.

this peculiar form up to that time have been discussed and they also suggest answers to these questions. They say: "The consistent and abundant production of purely matroclinous and constantly female offspring by this hybrid form of fish finds its most plausible explanation as parthenogenesis. It is not a spontaneous parthenogenesis, since many controls, unmated, have shown no indications of becoming pregnant. We provisionally assume that we are dealing with a case of gynogenesis (parthenogenetic development initiated by sperm which for some reason is prevented from taking part in heredity)—a condition recorded as naturally occurring among invertebrates, but not among vertebrates.



This hypothesis does not exclude alternatives and requires cytological verification."

With the above postulates in mind the writer started a series of investigations on the cytology of the parent species (*M. sphenops* and *M. latipinna*) and the unisexual female form, *M. "formosa"*, with the idea of finding an explanation for the reproductive phenomena evidenced by this hybrid form.

Various possibilities have been considered in this study. Among these are the following: (1) Parthenogenesis; (2) Gynogenesis; (3) Differential death-rate of the sexes; (4) The presence of some factor, acting during the period of sex differentiation, favourable to the development of females; (5) A possible explanation in sex inheritance studies reported for fish; and (6) An explanation offered by a possible atypical distribution of the maternal and paternal chromosomes during the maturation of the eggs of *M. "formosa"*.

MATERIALS AND METHODS

The materials for this study consisted of individuals from healthy stocks of the various species of *Mollienisia*. These stocks were reared in aquaria, the temperature being maintained in the upper seventies, rarely extending below 70° or much above 80° F. The food consisted of dried shrimp, live tubificid worms, and live *Daphnia*. They also fed on algae from the sides of the tanks. Besides these aquaria-reared specimens, several hundred individuals taken from their natural habitats were examined.

The techniques employed in the preparation of the materials varied. The fixatives used were: Bouin's, Allen's modification of Bouin's, Gilson's, and Petrunkevitch's cupric-phenol. The specimens taken from the natural habitat were fixed in formalin and preserved in 70% alcohol. Some of the tissues were dehydrated by the drop method and cleared and embedded by the xylol-paraffin method. Other tissues, mostly ovaries containing large amounts of yolk, were dehydrated and embedded according to the Dioxan method described by Mossman (1933). Most of the material, however, was dehydrated and embedded according to the normal-butyl alcohol technique developed by Stiles (1934). The tissues were sectioned in thickness ranging from 4 to 10 μ . depending upon their desired use. Various stains were used. Heidenhain's iron-alum haematoxylin (with picric acid in 96% alcohol used as the destaining agent) with or without a counter-stain, proved to be the most valuable for general structure. Ehrlich's haematoxylin was very satisfactory for yolk-filled

332 *The Reproductive Behaviour of Mollienisia "formosa"*

structures. Van Giesen's, Mallory's triple, and other stains were also used. As a counter-stain, Congo red gave the most favourable results, although good results were also obtained with eosin, Lichtgrün, and others.

I. PARTHENOGENETIC REPRODUCTION

Females possessing the power of producing offspring without sexual union with the male are said to be parthenogenetic. Among vertebrates there are no verified examples of the phenomenon occurring in nature. Morgan (1927), however, gave an adequate review of the researches of Bataillon, Harlant, Gunther & Paula Hertwig, Loeb, Morgan, and others which shows that parthenogenesis may be produced artificially in vertebrates.

For fish there is very little information concerning the production of young by artificial means. However, the evidence presented by Trifanowa (1931) indicates that the development of certain fish eggs without fertilization is possible. He was able to follow the development of eggs of *Acerina cernua* (after artificial stimulation) from early cleavage to the development of well-formed larvae, two of which hatched. He stated that sections through early segmentation stages showed ordinary karyokinesis. This paper is of importance, for it relates the first successful production of young by parthenogenesis among teleosts. Prior to this Bataillon (1904) had succeeded in producing development of eggs of *Petromyzon planeri* by artificial stimulation, but the eggs did not develop beyond the blastula stage. Newman (1915) says: "During eight years of work with fish eggs I have never had a single embryo develop in an egg that had not been intentionally fertilized."

Possible parthenogenesis in Mollienisia. When the male of any species of *Mollienisia* is mated with *M. "formosa"* the result is always the production of broods of young all of which are females. Parthenogenesis would offer an explanation for the production of broods of individuals all of which are of the same sex. Experiments performed and reported by C. L. & L. C. Hubbs, however, show that *M. "formosa"* females do not produce young without males being present. These results have been verified in the present investigation. Histological preparations of the ovaries of mature but virgin females further confirm this conclusion. Examination of several of these specimens showed not only the absence of young, but also that the ova contained in them had not reached the full size.

The problem presented by the requirement of the presence of males

in the production of purely female broods suggested that the males possibly serve merely as psychic influences which are needed to stimulate reproductive activity in these females. This hypothesis was tested by the following experiment.

Three aquaria were fitted with glass separations dividing them into right and left halves. Two *M. "formosa"* females were placed in the left half and two *M. sphenops* males in the right half of each of these tanks. The behaviour of the fish was watched closely for 5 weeks. The results obtained were as follows.

Very soon after the two sexes had been placed in the divided aquaria the males became aware of the presence of the females. The strong mating instinct of the males was obvious by their sustained effort to get beyond the glass plates separating them from the females. The activities of these males simulated that of the *Lebistes* males observed by Breder & Coates (1934). They reported that males isolated for 1 week reacted positively to females in the same aquarium and in an adjacent aquarium not more than 15 cm. distant; to anaesthetized females; to male specimens of *Cyprinodon variegatus*, *Fundulus heteroclitus*, and *Barbus conchoniensis*; and to the projected shadows of living fish on the sides of the aquarium. On the other hand, the *Mollienisia* "*formosa*" females did not display any sexual activity.

Two weeks after the beginning of the experiment one of the males, in aquarium 1, was found to have jumped over the partition into the section that contained the females. Because of this fact this aquarium was no longer considered a part of the experiment. The other two aquaria had very thick growths of duck-weed on the surface of the water which rendered it very unlikely that the males in either of these tanks ever came in contact with the females.

At the end of the 5 weeks not any of the females had given birth to young. Further, none of the eggs contained in their ovaries had developed to full size. Accordingly, this experiment proves that the males exercise more than a psychic influence in mating.

II. GYNOGENESIS

Possible gynogenesis in Mollienisia. In cases of gynogenesis the sperm penetrates into the egg but takes no part in the process of development. Most of the instances of this phenomenon have resulted from the penetration of eggs by sperm incapacitated by the application of external agents. Wilson (1925) included a review of the so-called "radium parthenogenesis" experiments of the Hertwigs in amphibians and

334 *The Reproductive Behaviour of Mollienisia "formosa"*

echinoderms. These experiments showed that sperm, rendered incapable of taking part in development through the influence of radium emanations, still stimulated eggs to develop, but that these eggs develop with only maternal chromosomes. Among the nematodes, however, two examples of gynogenetic inheritance have been found in which this method of development seems to occur normally. Eva Krüger (1913) found certain individuals of *Rhabdites aberrans* which looked like females, but were more than females, for they produced sperm as well as eggs, and were self-fertilizing. She found that true males also existed but were of uncommon occurrence. The eggs of these females produced only one polar body which seemed to indicate parthenogenesis of the diploid type. The eggs were regularly penetrated by the sperm, but the sperm degenerated *in situ* without having fused with the egg nucleus. Some of these eggs were found which developed without the sperm (no sperm nucleus being found), leaving the case uncertain as to the necessity of the sperm in initiating development. Paula Hertwig (1920) observed a mutant of *Rhabdites pellio*, a normally dioecious species, which produced only female offspring. She reported that the eggs of this form produced only one polar body and that development was accordingly diploid, but that development failed if the egg had not first been penetrated by the sperm. She was able to follow the unfit sperm nucleus in the dividing egg.

The similarity of the reproductive behaviour of *Rhabdites* to that of *Mollienisia "formosa"* suggests that in this form a like type of fertilization might exist. This idea of gynogenesis advanced by C. L. & L. C. Hubbs has been commented upon by Howell (1933) as follows: "It is unwise to introduce the suggestion of parthenogenesis, even of a modified sort, into vertebrate literature. The phenomenon is so at variance with what is known and believed about vertebrate development that I am sure no vertebrate morphologist would admit for a moment that the natural development from an egg to sexual maturity of an individual vertebrate without the direct inclusion of the male element is within the realms of probability. Certainly extremely convincing cytological evidence would be necessary and the experiments verified several times."

In normal fertilization of teleost eggs a fusion nucleus is formed and the resulting individuals should have the characteristics of both parents. However, through hybridization various modifications of normal fertilization might conceivably produce a gynogenetic effect. In all cases the sperm must penetrate the ovum, but the subsequent history of the two nuclei may be different. The sperm may penetrate the egg and die *in situ*

without union with the egg nucleus and so serve no other function than that of an exciting agent which stimulates the egg to development. Fish developed from such eggs would be expected to have the haploid number of chromosomes. They might also develop with a diploid number of chromosomes, if one of the polar bodies were retained, or if the chromosomes should be duplicated during early cleavage stages. In either case, the result would be development with only the hereditary factors derived from the maternal parent.

The sperm might also succeed in reaching the first cleavage spindle, but some of the paternal chromatin might be lost through lagging. Embryos derived from such eggs would show a dominance of maternal characteristics. Miss Pinney (1918) reported behaviour of this type in heterogeneric crosses in which she fertilized *Fundulus* eggs with sperm of *Ctenolabrus*. In the reciprocal cross, however, she showed that the paternal chromatin was not eliminated and stated that "early mitotic behaviour is prevailingly normal".

Time of fertilization and its relation to the problem of gynogenesis. In an investigation attempting to discover the mechanism of fertilization it is necessary to determine when fertilization occurs. Though several investigators have made studies of the reproductive systems of various viviparous teleosts, very little information has been gained concerning the mechanism of fertilization or the time when it occurs. Eigenmann (1892) observed the extrusion of the second polar body and the presence of the male pronucleus in an egg still surrounded by the cells of the follicle in *Cymatogaster aggregatus* Gibbons, but this was merely a chance discovery. Hildebrand (1917) noticed the presence of various generations of eggs at the same time in the ovaries of *Gambusia affinis* and *Cyprinodon variegatus*, but reported: "When the fertilization of the different sets of eggs occurs is not known." Bailey (1933) studied the ovarian cycle in *Xiphophorus* and attempted to establish a means of dating pregnancies, but his method did not indicate how the time of fertilization might be determined. He was unable to find fertilization stages.

In *Mollienisia* the length of time between the introduction of a male into an aquarium containing virgin females and the delivery of the first brood of young varies considerably for different individuals. The first brood may be produced in 4 or 5 weeks; in other cases months may elapse. An interesting example is found in the following information given to me by Mrs Carl L. Hubbs. A male was placed with a female 15 December 1932. On 15 May 1933 the male died, and at this time no young had been produced. The female was left alone in the aquarium,

336 *The Reproductive Behaviour of Mollienisia "formosa"*

and on 4 August 1933 she produced a brood of thirty-three young. The irregularities described above made it inadvisable to attempt to discover the time of polar body formation in ovaries taken from females which had not previously produced young.

Mollienisia, like several other poeciliid fish, may produce several successive broods of young after a single mating. This is possible because they have the capacity to store sperm. Philippi (1908) described stored sperm in the ovaries of *Glaridichthys januaris* and *G. decem-maculatus*.

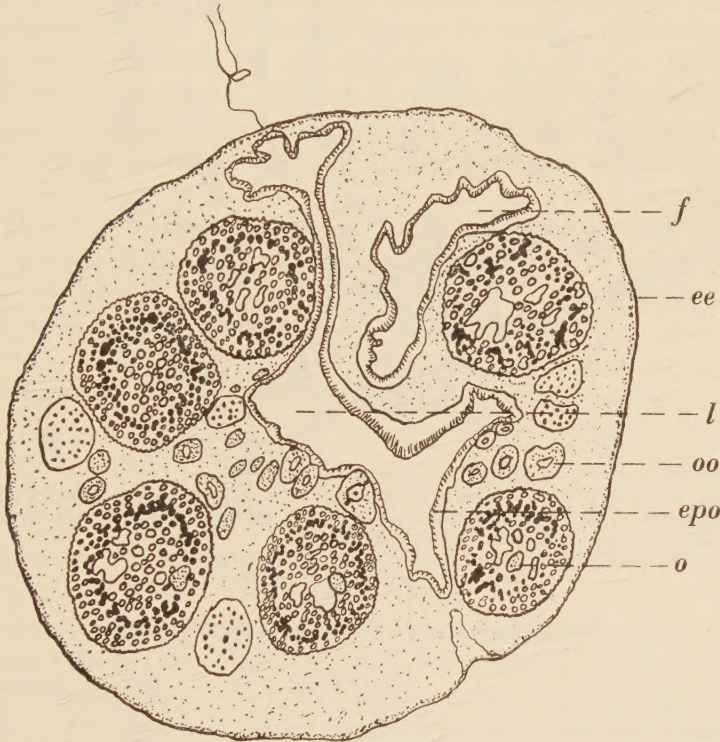
Further, when females are in the later stages of pregnancy the males show little interest in them. Shortly after a female has delivered a brood, however, the males exhibit a great increase in sexual interest. Active mating is a common occurrence at this time. These two facts—the capacity of the females to store sperm, and the mating activity following parturition—are perhaps responsible for the continued sexual activity of the females. In general after a female has produced her first brood a second brood follows. The length of time between the first and second broods gives approximately the duration of the gestation period. This has been found to vary for different individuals. For any one individual the time may vary for successive broods. From a study of records of many females the average interval has been found to be from 28 to 35 days.

These facts suggested that fertilization must take place soon after parturition. Accordingly, females were fixed at various intervals following their delivery of a brood. The results concerning the time of fertilization in *Mollienisia*, based on examination of preparations made from these ovaries, are contained in the following summary:

- (1) Ova in various stages of development are found within the same ovary (Text-fig. 2).
- (2) Sections of an ovary fixed shortly after parturition (4–6 hr.) show that the largest ova, which will form the next brood, are growing rapidly. This is evidenced by yolk production.
- (3) The size of the largest ova shortly after parturition varies for different individuals.
- (4) The size that the ova must attain before they are fertilized could not be determined. Eggs showing early cleavage stages are approximately 2 mm. in diameter. This indicates that the ova must be nearly that size before fertilization takes place.
- (5) Sperm are present in the ovary during all stages of the pregnancy period. They have been found at the bottom of the fertilization valley, closely lined against the follicular epithelium, as early as 6 hr. after parturition (Text-fig. 3).

(6) Ovaries fixed 1, 2, 3, and 5 days after parturition failed to show penetration by the sperm. Consequently, polar body formation has not been observed. A female fixed 9 days after parturition contained embryos approximately 4 mm. in length.

(7) These observations indicate that the metabolic condition of the female during the previous pregnancy determines the size the ova for



Text-fig. 2. Cross-section of a *Mollienisia* "formosa" ovary 6 hr. after parturition. Eggs of various stages may be seen at the same time. *ee*, external epithelium of ovary; *epo*, epithelium of ovarian cavity; *f*, spent follicle; *l*, ovarian cavity; *o*, ovum; *oo*, small oocytes.

the next brood attain by the time of parturition. Also that fertilization occurs after parturition, the time at which it occurs varying according to the length of time required for the ova to attain the full size.

(8) These conditions have made it impossible to determine, without further study, whether or not the purely matroclinous inheritance of the *M.* "formosa" hybrids is the result of the elimination of paternal chromatin.

Spermatogenesis and fin regeneration and their bearing on the problem of gynogenesis. The chromosome numbers of several teleosts have been



Text-fig. 3. Small portion of the ovarian cavity of a *Mollienisia "formosa"* 6 hr. after parturition. *epo*, epithelium of ovarian cavity; *fe*, follicular epithelium; *fv*, fertilization valley; *o*, ovum; *s*, sperm; *t*, theca of follicle.

TABLE I

Chromosome numbers of some poeciliid fishes

Species	Investigator	Date	No. of chromosomes
<i>Gambusia holbrooki</i>	Geiser	1924	36
<i>Lebistes reticulatus</i>	Winge	1922	46
<i>Lebistes reticulatus</i>	Vaupel	1929	46
<i>Lebistes reticulatus</i>	Iriki	1932	46
<i>Platyopocilus variatus</i>	Friedman & Gordon	1934	50
<i>Platyopocilus maculatus</i>	Friedman & Gordon	1934	48
<i>Platyopocilus xiphidium</i>	Friedman & Gordon	1934	48
<i>Xiphophorus helleri</i>	Ralston	1933	48
<i>Xiphophorus helleri</i>	Friedman & Gordon	1934	48
<i>Xiphophorus jallapa</i>	Ralston	1933	48
<i>Xiphophorus montezumae</i>	Friedman & Gordon	1934	48

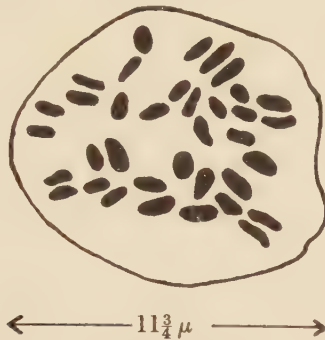
determined from studies of spermatogenesis. The numbers determined for various poeciliid fishes are found in Table I.

If the purely matroclinous inheritance of *M. "formosa"* is the result of the elimination of paternal chromatin, the number of chromosomes must be smaller in this hybrid form of fish than in the parent species.

In order to determine if this condition exists in these forms, tissues in which chromosome counts could be made were prepared.

Transforming males (males in which the gonopodium is just starting to develop) of the parent species, *M. sphenops* and *M. latipinna*, and males of *sphenops* $\times$ *latipinna* hybrids¹ were decapitated, the body cavity slit open, and then placed in the fixative while the heart was still beating. Slides from which chromosome counts could be made were prepared from such material. A complete account of spermatogenesis in these forms will be published in a separate article.

Winge (1922 a) found divisions in the ovary of *Lebistes* from which he obtained chromosome counts, but such counts have only occasionally been made in viviparous teleosts. He had, however, previously deter-



Text-fig. 4. Camera-lucida drawing of spermatogonial cell of *Mollienisia sphenops* showing thirty-six chromosomes.

mined the number in this form from studies of spermatogenesis. In the ovaries of *Mollienisia* "*formosa*" which were examined in this study no cells were observed in which chromosome counts could be made.

Counts by means of camera lucida drawings of spermatogonial and primary spermatocyte divisions show clearly that the diploid number of the parent species is 36 (Text-fig. 4). This result has been verified by frequent counts at different times and also by counts made by co-workers in the laboratory. It has been established beyond any doubt that the F_1 *M. sphenops-latipinna* male hybrids also carry the diploid number of chromosomes.

The fins of fish have long been used as material for regeneration studies and present a source of tissue where rapidly dividing cells may be found. A series of caudal fins of healthy individuals of the three types

¹ When *M. sphenops* and *M. latipinna* are hybridized in the laboratory both male and female offspring are produced.

340 *The Reproductive Behaviour of Mollienisia "formosa"*

of *Mollienisia* were clipped. The fins regenerated rapidly, and in a few days new tissue was quite abundant. After about 4 days the regenerated portion was removed, fixed and prepared for sectioning. These regenerated fin tissues were carefully examined, and some of the cells were found to be in division. The chromosomes, however, were, in general, greatly clumped, and this made it difficult to secure accurate counts. Nevertheless, the material was of value for it permitted a comparison of similar cells in somatic tissues of *M. "formosa"* with those of *M. sphenops* and *M. latipinna*. The nuclei of 100 cells in the regenerated tissue of each of the forms were measured by the aid of an ocular micrometer and the average size calculated. The average diameters in the three forms were as follows: *M. sphenops* 5.43μ ., *M. "formosa"* 5.47μ ., and *M. latipinna* 5.49μ . This similarity is also illustrated by photomicrographs of blood cells of these groups.

Such comparisons show clearly that the nuclei of similar cell types are of equal size in these forms. If nuclear size is dependent upon the number of chromosomes present, as is generally considered to be the case, the number should be at least very nearly the same in all three forms. Such evidence is against the possibility of *M. "formosa"* having a haploid (or a polyploid) number of chromosomes.

The results of the present studies of spermatogenesis and fin regeneration, when considered in relation with the findings of other investigators, give strong evidence for the assumption that when the eggs of *M. "formosa"* are fertilized by sperm of either *M. sphenops* or *M. latipinna* the paternal chromosomes are not necessarily eliminated. Instead, the resulting generation of *M. "formosa"* may develop with both maternal and paternal chromosomes, but the paternal chromatin may not be able to affect the nature of the offspring.

III. DIFFERENTIAL VIABILITY

When the factors which might account for the occurrence of purely female populations, where normally both males and females should be expected, are considered, the possible existence of a differential viability of the males and females is immediately brought to mind. The males, if less viable than the females, might die during embryonic, juvenile, or adult stages. For example, when *Drosophila melanogaster* females are mated to *D. simulans* males, only females are obtained. Sturtevant (1920) showed that among the offspring the dead and dying larvae were males. Bonnier (1924), working on the same problem, made a histological study of these males. He reported that death was due "to a suspension

of the normal synchrony between the development of the different organs. Certain parts of the organs in hybrid larvae may thus be found at a purely larval stage, while others have undergone complete metamorphosis."

Howell (1933) suggested that the occurrence of only females in *Mollienisia* "*formosa*" might "be ascribable in this instance, perhaps, to a sex-linked lethal factor acting on male eggs, or possibly some type of non-disjunction". In this connexion he made mention of the fact, "that a yellow (mutant ?) strain of *Xiphophorus*, developed in domestication, consistently produces three or four times as many females as males, and that the case of the *Mollienisia* may be an instance of this factor carried to the extreme".

Three types of evidence have been used in this investigation to test this hypothesis: (a) genetic, (b) histological, and (c) studies in fertility.

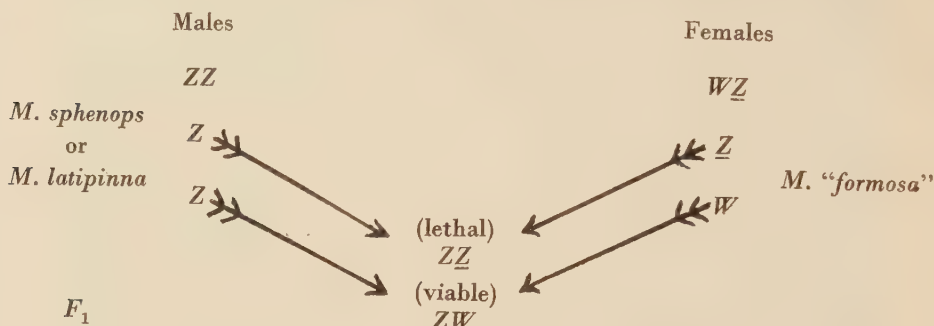
Genetic evidence. If a sex-linked factor lethal to the males exists, only females should be born. Males of *M. sphenops* or *M. latipinna*, when mated with the *M. "formosa"* produce only female broods; when mated with females of their own kind they produce males and females in the normal ratio. This suggests that the *Mollienisia* females may be heterogametic for sex, and that a factor lethal to the males may be carried in the Z-chromosome of *M. "formosa"*. Depending upon chance distribution, half of the mature eggs should carry the W-chromosome and half the Z-chromosome. The eggs would all be fertilized by sperm carrying a Z-chromosome. The eggs containing a Z-chromosome, fertilized by a sperm also containing a Z-chromosome, should produce males. The eggs containing a W-chromosome, fertilized by sperm containing a Z-chromosome, should produce females. An egg containing the Z-chromosome with its factor lethal to the males, would not reach the birth stage, whereas an egg with a W-chromosome would. This would result in a production of purely female broods. In Text-fig. 5 the chromosomes assumed to carry the lethal factors are underlined.

This means that the Z-chromosomes of the new female generation of *M. "formosa"* were received from the male parent. If they are now back-crossed with a male parent, a reunion of two male-parent type Z-chromosomes would take place. Unless the Z-chromosomes of the male-parent type have been modified by the action of some agent (W-chromosome, autosome, cytoplasm, etc.) in *M. "formosa"* they should not produce an effect lethal to the males of this generation. Both males and females should be produced as shown in Text-fig. 6. In the case of *M. "formosa"* this has not been found to be true. These females

342 *The Reproductive Behaviour of Mollienisia "formosa"*

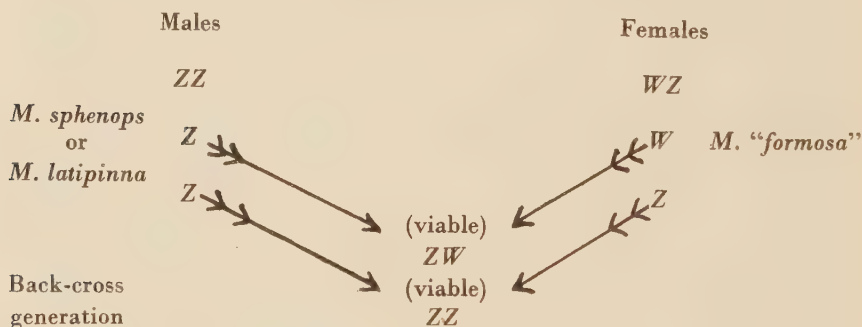
have been back-crossed with the males of *M. sphenops* and *M. latipinna* for several generations and no males have ever been born.

If we consider the *Mollienisia* males as being heterogametic for sex, the same type of evidence can be presented. A sex-linked lethal factor might account for the production of female broods for one generation, but not for several generations. Further, the production of purely female broods, by *M. "formosa"*, by the action of lethal factors does not



Text-fig. 5. Chromosome behaviour in *Mollienisia* if a sex-linked lethal factor is assumed.

Note. This explains the purely female broods for one generation only.



Text-fig. 6. Chromosome behaviour in *Mollienisia* if a sex-linked lethal factor is assumed.

Note. The lethal factor has been lost.

offer a solution to the problem of matroclinous inheritance. The fact of existence of female broods alone is not sufficient. To explain the reproductive behaviour of *M. "formosa"* one must show not only why all of the offspring are females but also why none of the characters of the male parent involved is exhibited by these female offspring. The lethal factor hypothesis does not meet these requirements.

Histological evidence. Numerous broods of *M. "formosa"*, produced in the laboratory, have been reared to the adult stage without the loss

of any individuals. This eliminates the possibility of a differential viability acting after birth, though it tells us nothing about conditions during embryonic development. In order to determine if degenerating ova or embryos might be present during prenatal stages about 500 females were dissected, and sections were made of some of the ovaries and young in various stages of development. In this material no degenerating ova or embryos were found. Occasionally a few full-sized unfertilized ova were present in ovaries containing well-developed embryos, but such were also observed in ovaries of *M. sphenops* and *M. latipinna*. A few of the ovaries contained embryos which were not so well developed as the other embryos of the brood. However, they were apparently normal and did not represent eggs which would develop into males. They were probably ova which had been fertilized somewhat later than the others. One ovary contained three embryos of hatching size, along with other ova which had not quite the full complement of yolk. This existence of embryos not in step with the other members of the brood explains the birth of the occasional young 2 or 3 days after the main part of the brood has been delivered. A careful and complete study of the reproductive systems of these females showed no evidence of degenerating ova or embryos.

Studies in fertility. Fifty females of each of the forms, *M. sphenops*, *M. latipinna*, and *M. "formosa"*, were examined, and the number of young contained in each determined. Pregnant females were selected from collections taken from the natural habitats of these forms. Each specimen was measured according to the standard measurement (tip of the maxilla to the tip of the caudal peduncle), and after dissection of the ovary the embryos were counted. The results of these determinations are found in Table II. Though the number of individuals that has been examined is not large, it is considered representative. The figures show that no great differences in the fertility of these forms is found in nature. There is a slight difference which indicates that *M. latipinna* is the most and *M. sphenops* the least productive, and that *M. "formosa"* is intermediate between the two. C. L. & L. C. Hubbs¹ have found that under laboratory conditions the order is reversed, and that *M. sphenops* is the best producer, followed closely by *M. "formosa"*, while *M. latipinna* has been found to be very difficult to rear, generally producing very small broods.

The length of the smallest *M. "formosa"* found to contain young was 29 mm., and that of the largest 66 mm. The sizes of the broods ranged

¹ Unpublished records.

TABLE II

Comparative fertility of three groups of Mollienisia

<i>M. sphenops</i>		<i>M. "formosa"</i>		<i>M. latipinna</i>	
Size of ♀	No. of young	Size of ♀	No. of young	Size of ♀	No. of young
72	60	66	105	60	80
68	45	58	59	58	84
65	48	55	83	58	64
63	44	55	28	58	56
62	117	55	24	56	50
60	24	55	21	55	57
59	41	53	73	55	50
59	25	53	52	55	45
58	53	53	32	55	37
57	42	52	83	54	51
57	33	52	47	53	65
56	40	52	41	53	20
56	36	52	38	52	57
54	40	52	33	52	52
54	27	52	24	52	50
53	32	50	42	52	46
51	38	50	30	51	30
51	35	50	30	51	30
51	22	50	22	50	47
49	37	49	42	49	39
49	22	49	28	49	20
48	36	48	53	48	55
48	25	47	31	48	42
48	17	47	20	48	39
47	26	46	40	48	38
46	17	45	31	47	41
45	37	45	23	47	26
45	31	45	16	46	43
45	22	45	14	45	31
45	13	44	34	45	23
44	27	44	20	44	33
44	9	42	17	44	28
43	24	41	14	44	27
43	14	40	20	42	41
42	23	40	19	42	26
41	21	40	5	40	26
40	13	39	13	40	20
39	26	38	21	38	18
38	15	38	18	37	15
38	14	38	14	35	19
38	10	38	8	33	10
38	9	37	15	32	16
37	12	37	13	32	11
36	11	35	18	32	10
36	11	35	9	31	18
36	10	32	11	31	14
36	10	32	11	31	11
36	9	30	11	30	11
35	7	30	6	30	12
32	9	29	9	29	9
Av. 47.8	27.4	45.2	29.8	45.8	34.8

Size = length in mm.

from five to 105. These facts show that this form is mature as early as *M. latipinna* and *M. sphenops*, and further, that it is capable of producing broods which compare favourably with those produced by them. All of the facts obtained from this study in fertility of these forms speak against the elimination of the *M. "formosa"* males by a differential viability.

The above three types of evidence, a genetic analysis, a histological study of the female reproductive system, and a study of the fertility of *Mollienisia*, indicate that the production of only females by *M. "formosa"* is not due to an elimination of the males in embryonic or adult stages.

IV. SEX DIFFERENTIATION AND FEMALE POPULATIONS

General considerations. Huxley (1920) suggested that several instances of peculiar sex ratios among fish might be due to the fact that some of the individuals within certain populations had undergone sex reversal. Essenberg (1925), Harms (1926), Kosswig (1933), and Friesz (1933) observed changes in the sex ratios of *Xiphophorus* stocks, and all have explained that the change was due in part to reversal of some of the individuals from females to males. Essenberg and Friesz described the histological changes in the gonads which accompany sex reversal in these forms. Blacher (1926) described a reversal of sex from male to female in *Lebistes*, and described the histological picture of a hermaphrodite gonad. Eggert (1931) noticed in *Periophthalmus vulgaris* a great preponderance of females which represented progressive phases of sex reversal in the male direction. He called the condition "transitorische Intersexualität". Among the not yet sexually mature females he observed that a portion of the largest oocytes regularly degenerated before the beginning of yolk production. Later (1933), he described in great detail the histological structure in the gonads of these intersexes.

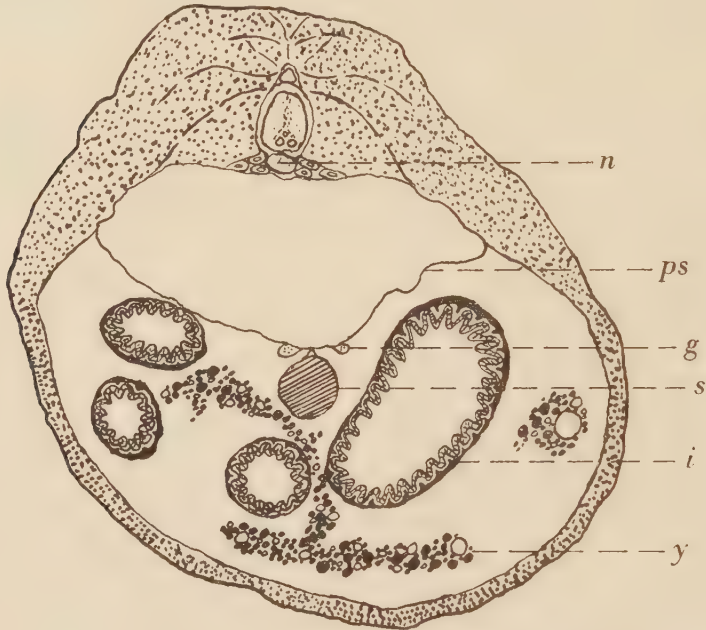
These observations of other investigators suggested the idea that the purely female broods of *M. "formosa"* might be the result of some factor acting during the period of sex differentiation. To test this the ovary was studied during various stages of development. To make possible the recognition of male characteristics, should any be present, a study was also made of sex differentiation in *M. sphenops* and in *M. sphenops-latipinna* hybrids produced in the laboratory, a group having both males and females. Over seventy-five embryos, young fish, and adults have been sectioned in this phase of the study.

The history of the germ cells and stages of sex differentiation of various viviparous poeciliids have been described by investigators

346 *The Reproductive Behaviour of Mollienisia "formosa"*

working with other forms: Essenberg (1923) and Friesz (1933) in *Xiphophorus*, Wolf (1931) in *Platypocilus*, and Goodrich *et al.* (1934) in *Lebistes*.

In the present study of sex differentiation no attempt has been made to trace the early history of the germ cells or to give a detailed history of the development of the reproductive system of *M. "formosa"*. The aim has merely been to investigate the possible existence of juvenile hermaphrodites in this form.



Text-fig. 7. Transverse section of a 9 mm. *Mollienisia "formosa"* embryo. *g*, genita lridge; *i*, intestine; *n*, notochord; *ps*, peritoneum of swim bladder; *s*, spleen; *y*, yolk.

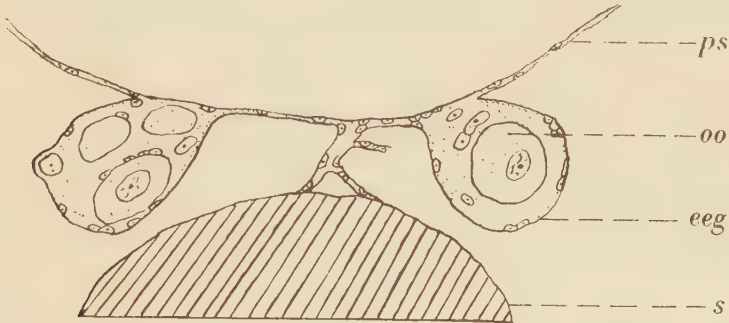
Sex differentiation in M. "formosa"

(a) 5 mm. embryo. Embryos of this size are prenatal and are secured by dissecting them from the ovary of the parent. They still have large yolk-sacs, around which the embryos are coiled and confined within the egg membranes.

The stage may be defined as the early genital ridge stage, and the germ cells are confined to two interrupted longitudinal ridges lying below the swim bladder. These early gonads are made up of the following types of cells: the large primordial germ cells ($5-8\mu$. in diameter); loose stromal

cells irregularly distributed among the germ cells; and peritoneal cells which surround the gonad fundaments.

(b) *9 mm. embryo.* Embryos are of this size on the day of birth. The yolk-sac has disappeared, but cross-sections show the presence of large quantities of undigested yolk (Text-fig. 7). The gonad anlagen are still paired but extend much farther into the coelom than they do in the 5 mm. stage and are closer together. The germ cells exhibit the first signs of change in a female direction in that some of them have attained



Text-fig. 8. Genital ridges of the 9 mm. *Mollienisia* "formosa". eeg, epithelium of genital ridge; oo, oocyte; ps, peritoneum of swim bladder; s, spleen.



Text-fig. 9. Genital ridges of the 12 mm. *Mollienisia* "formosa". eeg, epithelium of genital ridge; oo, oocyte; ps, peritoneum of swim bladder; s, spleen.

a diameter of 20μ , and give all indications of being young oocytes (Text-fig. 8). The stromal and peritoneal constituents are like those in the 5 mm. embryo.

(c) *12 mm. embryo.* Embryos attain this size about 4 days after birth. At this stage all the yolk has been digested. The two germinal ridges are beginning to fuse along the mid-line and the gonad can definitely be identified as an ovary (Text-fig. 9). Some oocytes have attained a diameter of about 30μ . The ovarian cavity is beginning to form. The way in which this cavity develops from paired primordia has

348 *The Reproductive Behaviour of Mollienisia "formosa"*

been described by Essenberg and Friesz in *Xiphophorus*. The method of its formation is the same in *Mollienisia*.

(d) *15 mm. embryo*. In embryos of this size the two primordia are completely fused, and the invagination of the ovarian peritoneum to form the ovarian lumen is very marked. Numerous young oocytes, some of which are surrounded by a follicle of a single layer, are present. Some of these are about 40μ . in diameter. Numerous oogonial spiremes are present and indicate rapid multiplication (Text-fig. 10).

(e) *Young female, 21 mm.* The ovarian cavity is completed and few traces of a bilateral origin are present (Text-fig. 11). The ovary has



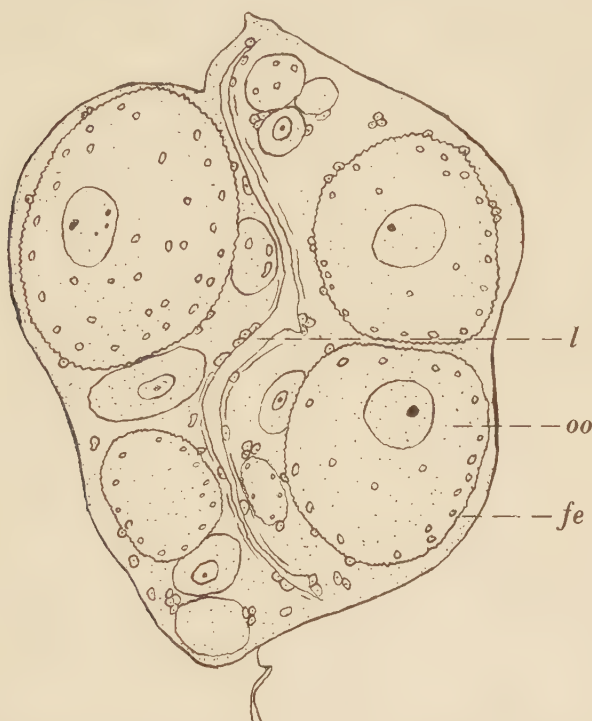
Text-fig. 10. Ovary of the 15 mm. *Mollienisia "formosa"*, showing the union of the two genital ridges and the increase in the number of the oocytes. *bv*, blood vessel; *eeg*, epithelium of ovary; *og*, oogonial cell; *oo*, oocyte; *oof*, oocyte follicle; *ps*, peritoneum of swim bladder; *s*, spleen.

increased markedly in size due principally to the growth of the oocytes, some of which are now 0.12 mm. in diameter. Very little stroma is present, since many of the stromal cells have been used up in the formation of the follicles around the oocytes, and many have been incorporated with the epithelial wall of the ovarian cavity. Wolf (1931) found the same to be true in *Platypoecilus*.

(f) *Adult virgin female, 33 mm.* Ovaries of adult virgin females are different from those of (e) only in the increased number of large oocytes. Some of the larger oocytes show clearly early stages of secondary yolk development and have attained diameters of about 0.50 mm.

Conclusion. In this study of the development of the ovary of *M.*

"*formosa*" no individuals have been found which show any tendencies toward juvenile hermaphroditism.



Text-fig. 11. Ovary of a 21 mm. *Mollienisia* "*formosa*", showing the single ovarian lumen resulting from the union of the two genital ridges. *fe*, follicular epithelium; *l*, ovarian cavity; *oo*, oocyte.

V. SEX DETERMINATION AMONG FISH

Sex chromosomes have not been demonstrated cytologically in fish. However, genetic studies have shown that in some groups it is the male, in others the female that is heterogametic, while in still others heterogamety has been shown to be non-existent.

(a) *Heterogametic males*. Aida (1921) in his studies of inheritance in *Aplocheilus latipes* demonstrated that the males were heterogametic and that a gene for red body (**R**) was carried both by the *X* and *Y*-chromosomes. He also showed that crossing-over of the **R** gene between *X* and *Y*-chromosomes was not a rarity.

One of the most extensive studies of inheritance in fish has been made by Winge in *Lebistes reticulatus*. In his early studies (1922 *a, b*) he showed that the males were heterogametic and that factors were present

in both *X* and *Y*-chromosomes. In 1923 he reported crossing-over, a factor "e" for *elongatus* being transferred from the *X* to the *Y*-chromosome. Later (1927), he located nine genes in the *Y*-chromosome and three in the *X*-chromosome, and found five genes that showed crossing-over in various ways from *X* to *Y*, from *Y* to *X*, and from *X* to *X*, since crossing-over takes place in both sexes.

In 1930 he observed that in certain *Lebistes* races some of the females developed male characteristics, e.g. colour. By appropriate crossings he obtained a few of the so-called *XX* males which he mated to normal females and obtained only female offspring. Aida (1930) also reported males which when mated to any normal female produced mainly female offspring, but considered his males as cases of non-disjunction-*XXY* males. Winge (1930), however, expressed the opinion that Aida's *Aplocheilus* males, "must be regarded not as having arisen through non-disjunction, but as being *XX* males". Crew (1921) recorded a similar condition in frogs in which an individual, though originally a female, exhibited the complete organization of the male and functioned as a male. Such an individual fertilized the eggs of a normal female, and all of the 774 offspring which attained a sufficient development to permit the identification of sex were females.

Recently, Winge (1932) presented evidence that sex chromosomes in *Lebistes* may be changed experimentally into autosomes and vice versa, and reported that it is theoretically possible within a species to change from male to female heterogamety.

(b) *Heterogametic females.* *Platypoecilus* also is a genus of the family Poeciliidae. Like *Lebistes*, it has proved to be an excellent form for laboratory studies of inheritance. Bellamy (1922 *a, b*) reported sex-linked inheritance in this group. Unlike *Lebistes* the females of *Platypoecilus* have been found by Bellamy (1928) and also by Fraser & Gordon (1928) to be heterogametic. Fraser & Gordon (1929) showed that the *W*-chromosome usually bears recessive genes but that crossing-over occasionally occurs between the *W* and *Z*-chromosomes so that dominant genes of the *Z* interchange with their recessive allelomorphs in the *W*-chromosome. This transference of dominant genes from the *Z* to the *W*-chromosome gave rise to females which exhibited one-sided feminine inheritance.

(c) *Phenotypic sexuality.* The investigations of various workers on *Xiphophorus*, a third genus of poeciliids, have not definitely established heterogamety in this group. Harms (1926) observed instances of sex reversal in this genus and mated some of the masculinized females with

normal females. Because he obtained purely female broods from these females he concluded that the males were heterogametic. Later in the same year, Essenberg (1925) also reported complete sex reversal in this group but his studies led him to state that "Chromosomes are not considered as having the function of sex determination and control... Sex is determined and controlled by the sex hormones derived from the ovary and testis." Friesz (1933) arrived at a similar conclusion: "Die Geschlechterverteilung erfolgt hier also nicht durch einen Heterochromosomenmechanismus, sondern wahrscheinlich durch einen Modus der dem bei Hermaphroditen gleicht."

Kosswig (1933) attempted to determine the type of sex-determining mechanism of this group by mating *Platypoecilus* males, genotypically sexual (ZZ), to *Xiphophorus* females. From such crosses he obtained male and female hybrid offspring. Previously, Gerschler (1914) had succeeded in making intergeneric crosses between *Xiphophorus* and *Platypoecilus*, and explained his results according to Goldschmidt's (1933) theory of sex determination. Kosswig, however, concluded that Correns' theory of sex determination fitted his facts better than Goldschmidt's.

According to Kosswig's interpretation, *Xiphophorus* is a latent hermaphrodite in which not one of the two sex-factor groups *M* or *F*, taken in Goldschmidt's sense, but the anlagen of the sex organs as a whole are acted upon by outside influences. These anlagen or anlagen complexes are inherited by the hermaphrodite *Xiphophorus* as well as by the genotypically determined *Platypoecilus*. According to this theory, in genotypically determined groups, there is a single realizer, namely, that for the heterogametic sex. If this realizer fails, then the other sex develops, simply through the exceptional receptivity of the anlagen complexes for influences leading to the development of organs of the opposite sex, without other realizers being necessary to produce it. In groups without a heterochromosome sex-determining mechanism (phenotypically sexual), sex becomes realized only through the working of outside influences upon the gonad anlagen. Thus one of the two sexes is established during early development because influences which produce that sex exert a sufficient influence upon gonad anlagen at this time. When these primary sex-determining events have expired, the maintenance of the determined sex is provided for through the production of sex hormones by the gonads. These sex hormones are responsible for the morphological expression of the undeveloped sex characters, which the poeciliids exhibit with particularly noteworthy diversity. Thus the

demonstration of sex hormones is essential to the theory and becomes of particular significance.

(d) *Hermaphroditism and sex hormones.* As early as 1881 McLeod described the hermaphroditic conditions in *Serranus*, *Sparides*, and *Sargus*. Since that time, numerous instances of hermaphroditism among teleosts have been reported, e.g. Southwell (1902), Fowler (1912), Turner (1931), and many others. Because they have, for the most part, only aimed to describe the morphology of the glands made up of both ovarian and testicular tissue they are not considered as being important for this discussion.

The cases of hermaphroditism in *Fundulus* reported by Newman (1908) and Chidester (1917), however, are important, for they throw some light on the influence of sexual secretions upon the secondary sex characters. In the case described by Newman, "the presence of a comparatively minute amount of imperfect testicular tissue had the negative effect of inhibiting in an individual, predominantly female, the transformation of juvenile into female color pattern; and the positive effect of producing in this individual, an approximation of male coloration and behavior". In contrast to these findings Chidester reported that "an appreciably large percentage of ovarian tissue scattered through the body cavity of a fish has no power to influence the formation of the secondary sex characters". From this comparison he concluded that "we must recognize the evidence for an internal secretion produced by a regularly formed organ but absent in diffuse ovarian masses".

Van Oordt (1925) studied the relation between the development of secondary sex characters and the structure of the testis of *Xiphophorus*. He recognized the production of sex hormones but concluded that it is improbable that the gonad contains groups of cells which are the specific source of the sex hormones.

Zahl & Dwight (1932) studied the effects of gonadectomy on the secondary sex characters in *Amia calva*. They concluded that the development of the nuptial livery in this male is governed by some principle, presumably of an endocrine nature, which is elaborated by the testis. Further, that though a caudal ocellus is potentially present in females, its appearance is inhibited by an endocrine secretion elaborated by the ovary.

(e) *External agents effecting sex determination.* The experiments of Mršić (1923) and of Huxley (1923) showed that late fertilization changed the sex ratio of trout. Mršić reported that eggs fertilized 4-7 days late resulted in an increase in the number of females, while those fertilized

21 days late resulted in an increase in the number of males. Huxley reported that eggs fertilized 4-7 days late caused an increase in the number of males but explained this discrepancy on the grounds that Mršić had worked with the rainbow trout, while he had worked with the brown trout. Both observers noticed a transformation of sex during development.

Mršić reported that if in an otherwise male gonad the germ cells ceased increasing in number and increased in size, an ovary was obtained. Conversely, in a female gonad, if the number of cells increased instead of the size of the cells, the gonad developed into a testis. In this connection the hermaphrodite trout described by De Beer (1924) is of interest. The trout was 60 mm. long and had a normal testis on the one side, while on the other side there was an ovo-testis in which the two kinds of tissue were in intimate association. He reported that the egg from which this fish developed was not a case of late fertilization.

(f) *General conclusions with regard to sex determination in fish.* Judging from the results of sex-determination studies of numerous investigators it appears that the general conclusions may be drawn that possibly all, or nearly all fish, carry the potentialities of both sexes, and that the establishment of a particular sex is dependent upon various factors, some of which are hereditary, while others are environmental. The inherited sex potentialities may be acted upon by influences outside the germ cells. In some forms the inheritance of these sex potentialities have been explained by the assumption of a heterochromosome mechanism, while in other groups it has been proved that such a mechanism is non-existent. In either event the sex of the individual is not irrevocably determined at the time of fertilization. This is exemplified by the numerous instances of sex reversal, resulting in the occurrence of individuals having the appearance and function of one sex but with the chromosomal constitution of the other. The factors acting outside the germ cells which might influence its development are likely to be of two types: (1) those acting within the individual, e.g. hormones, (2) those acting outside the individual, e.g. light, temperature, and pressure.

VI. INHERITANCE IN *MOLLIENTISIA* "FORMOSA"

(A) *General considerations.* From the above review of sex determination among fish, it is evident that the type of inheritance which *M. "formosa"* exhibits cannot be explained according to existing theories. In this form all of the offspring from any mating are females showing only maternal characteristics. Because of this, a theory that explains

the consistent production of females by this group must also demonstrate why the paternal characteristics, e.g. number of rays in the dorsal fin and other similar somatic characters, are absent in the offspring. Since the present investigation has shown that the production of only females by *M. "formosa"* does not seem to be the result of gynogenesis or of the elimination of the males during some stage of development, a theory which I shall speak of as "Selective Maturation" seems to offer an alternative solution to the problem. This theory implies an arrangement of the chromosomes on the meiotic spindle, in such a way that certain groups of chromosomes are inseparably bound together and act in heredity as though they were linked. In order to determine the applicability of this theory to the present case it seems advisable to consider the following related problems: (1) the relation of maternal and paternal influences in the development of fish hybrids, (2) cytoplasm and matroclinous inheritance, and (3) other cases of selective maturation.

(1) *Maternal and paternal influences in the development of fish hybrids.* In general, the early hybrid larvae produced by heterogeneric crosses among teleosts have shown a tendency toward a one-sided development in the maternal direction. Moenkhaus (1904) showed that when crosses are made between *Menidia notata* and *Fundulus heteroclitus*, species whose cleavage rates differ, the sperm did not modify the cleavage tempo of the egg. By cytological study he was able to follow the paternal and maternal chromosomes during the early cleavage stages and showed that the paternal chromatin was not eliminated. Loeb (1913) repeated the experiments and he also concluded that the cleavage tempo of the egg was not affected by a foreign sperm. He considered these maternal type larvae as pure breeds of the female species and not as hybrids. He says: "All the facts known about heterogeneous hybridization point to one conclusion; namely that in these cases fertilization is really a case of artificial parthenogenesis." Miss Morris (1914) studied the behaviour of the chromatin in hybrids of *Fundulus* and *Ctenolabrus* and reported that the foreign chromosomes lagged behind the others in going to the poles. However, all the paternal chromosomes were finally included in the daughter nuclei of the two-cell stage and there was no evidence of the elimination of paternal chromatin at any stage.

Günther & Paula Hertwig (1914) made a similar study of the hybrids of *Gobius joso* and *G. capito*, and also showed that the paternal chromatin was not eliminated. Among their hybrids they noticed several pathological forms which they considered to be the result of a disharmony between certain factors in the egg and sperm, and they advanced the

idea that the success of a cross between different species depends upon the degree of harmony between the maternal and the paternal idioplasm, and the interaction of the idioplasm of the sperm and the cytoplasm of the egg.

Newman (1915) found that a preponderance of maternal type larvae resulted from fertilizing *Menidia menidia notata* eggs with sperm of *Fundulus*, *Paranotus*, and *Gasterosteus*. He expressed the opinion that "even in larvae, however, that are pure or nearly pure maternal, there is doubtless carried a latent recessive in the germ plasm, the full complement of paternal inheritance factors, that would segregate out in the following generation, if it were possible to interbreed F_1 hybrids". In accordance with this view he concluded that in heterogeneous crosses, "we are not dealing with a case of parthenogenesis but with one involving a more or less complete dominance of the egg over the sperm".

Miss Pinney, as has previously been stated, reported that when eggs of *Fundulus* were fertilized by *Ctenolabrus* sperm, the early cleavage stages showed abnormal mitotic behaviour and elimination of paternal chromatin, while when the reciprocal cross was made it was prevailingly normal. She also observed abnormal nuclear behaviour when eggs of *Stenotomus* and *Menidia* were fertilized with sperm of *Ctenolabrus*, but that when the reciprocal crosses were made it was normal. From these observations she concluded that the development of fish hybrids depends "first upon the effect of the cytoplasm (of the egg) on the sperm, and second upon the reaction between the two germ nuclei—a third factor upon which the first two depends, namely the variable specificity of the effect of the cytoplasm toward the foreign spermatozoon".

Miss Guthrie (1925) suggested that "it may be possible to explain the development of hybrids on the assumption that the cytoplasm of the egg does not contain the materials which are necessary for the supply of foreign chromatin, or that the use of cytoplasmic reserves for the synthesis of foreign chromatin robs the egg of sources of energy necessary to complete development".

(2) *Cytoplasm and matroclinous inheritance.* Matroclinous inheritance has been explained in several larval hybrids by demonstration of the elimination of all or some of the paternal chromatin during early stages of development. Other hybrids of the maternal type have the full sets of paternal chromosomes indicating that the early embryonic stages are determined by the egg. It takes some time before the paternal factors, introduced by the spermatozoon, produce an effect. A few examples will serve to illustrate this point.

Toyama (1912) mated various races of silkworms and observed that when a female of a race having a dominant factor for egg colour "D" was crossed with a male of a race having a recessive factor for egg colour "R" all of the F_1 had the appearance of the dominant race. When he made the reciprocal cross, however, all the F_1 had the appearance of the recessive race. He mated the F_1 of both crosses *inter se*, and obtained dominants and recessives in a 3 : 1 ratio in the F_2 . His conclusions concerning this behaviour were that some of the characters of the offspring were predetermined by the maternal genes of the egg before fertilization; and that dominant factors introduced by the sperm are not potent in the immediate offspring but produce their effect in the next generation.

A similar explanation has been given by Sturtevant (1923) for the inheritance of dextral and sinistral coiling of certain gastropods. In this group sinistral females will produce only sinistral offspring, even though their eggs are fertilized by sperm bearing a dominant factor for dextral. Similarly, if dextral females are fertilized by sperm from a sinistral type, all of the offspring are dextral. In both instances the F_2 are wholly sinistral or wholly dextral, depending upon the appearance of the F_1 parent. In the F_3 both dextral and sinistral types appear. Sturtevant concluded that in these forms the paternal chromatin does actually produce an effect but that the effect is delayed for one generation.

The recent heterospermic merogony experiments by Hörstadius (1932) suggest that in some instances the cytoplasm is able to override nuclear influence. The spines of the plutei of *Paracentrotus lividus* are narrow at their ends, while those of *Echinus microtuberculatus* broaden out and are provided with hooks and protuberances. Hörstadius fertilized denucleated eggs of *Paracentrotus* with sperm of *Echinus* and, reciprocally, denucleated *Echinus* eggs with *Paracentrotus* sperm. In both instances he obtained plutei of the *Echinus* type, though the hooks of the plutei, developed from *Echinus* cytoplasm and *Paracentrotus* nuclear material, were rather small. He considered these experiments as important evidence of the fact that inheritance can in part be transmitted through the cytoplasm.

An excellent illustration of matroclinous inheritance among plants has been described by Goodspeed & Clausen (1917). They crossed two varieties of tobacco, *Nicotiana sylvestris* and *N. Tabacum* and observed that the F_1 hybrids were expressedly like individuals of the maternal species, namely, *N. Tabacum*. When they back-crossed these F_1 hybrids

with *N. sylvestris*, the offspring were of the *N. sylvestris* type, though a few aberrant forms were obtained. When back-crossed with *N. Tabacum* only *N. Tabacum* forms resulted. Both the back-cross types produced pure breeds when fertilized *inter se*. In explanation of this occurrence Morgan (1927) suggested that a single set of foreign chromosomes was not able to affect the nature of the offspring in the presence of a single set of chromosomes of the maternal species.

(3) *Cases indicating selective maturation.* Cleland (1926) observed a case of selective maturation in his study of meiosis of the pollen-mother cells of *Oenothera biennis* and *O. sulfurea*. He reported that synapsis does not occur in these forms but that the chromosomes arrange themselves in two circles on the metaphase plate of the reduction division, in such a way that all the maternal chromosomes go to one cell and all the paternal to the other. Cleland has indicated the significance of this behaviour as follows: "If pairs of homologous chromosomes are represented by AA' , BB' , CC' , etc., and if they are arranged within two circles in the following manner: $AA' BB' CC' DD'$ and $EE' FF' GG'$, then if chromosomes $ABCD$ are attracted to the same pole as EFG in one cell, they will also be attracted in all normal pollen mother cells, and $A'B'C'D'E'F'G'$ will pass to the other pole. Only two chromosome complexes will be formed, and these will be the same as the complexes which united to form the plant."

A similar behaviour of chromosomes has been described by Schrader (1921, 1923) in the meiosis of the homopteran *Pseudococcus nipae*. In this form there are five pairs of homologous chromosomes. One of each pair carries a certain amount of sex chromatin. He reported that the presence of the sex chromatin does not influence the behaviour of the chromosomes during maturation, but its presence does prevent a haphazard distribution of them. In this form the five sex chromosomes tend to remain clustered and therefore always go to the same pole, while the other five always go to the opposite pole.

A less clear-cut demonstration of peculiar meiotic behaviour has been reported by Metz *et al.* (1926) in the dipteran *Sciara*. In this form they observed the elimination of what they considered an "ordinary chromosome" as the result of an unequal segregation of the chromosomes and an asymmetrical cell division. Later, Metz (1931) reported: "It seems probable that this process is in some way connected with sex determination and that the 'ordinary chromosome' which is eliminated is a sex chromosome." Since all of the offspring are of the same sex he believes that the eliminated chromosome is possibly the paternal X.

The observations of these investigators indicate that in hybridization the following conditions may be involved: (a) The successful development of the hybrid depends upon the effect of the cytoplasm of the egg on the sperm and a harmonious interaction of the germ nuclei. (b) Some instances of matroclinous inheritance have resulted from elimination of the paternal chromatin in early cleavage stages, while others have resulted from an incapacity of a single set of foreign (paternal) chromosomes to produce an effect on the offspring in the presence of a single set of maternal chromosomes. (c) In the meiosis of hybrids the chromosomes may arrange themselves in the metaphase plate of the reduction division in such a way that only two chromosome complexes will form, and these will be the same as the complexes of the maternal and paternal species which joined to form the hybrid.

(B) *Selective maturation and its application in the reproductive behaviour of Mollienisia "formosa"*. C. L. & L. C. Hubbs assumed that *M. "formosa"* is a form produced by natural hybridization between *M. sphenops* and *M. latipinna*. It possesses nearly all the characteristics of a natural species, i.e. constant pure line, a definite range, and the ability to maintain itself in the struggle for existence. Accordingly, in the following discussion *M. "formosa"* is considered as a distinct form and not as a hybrid.

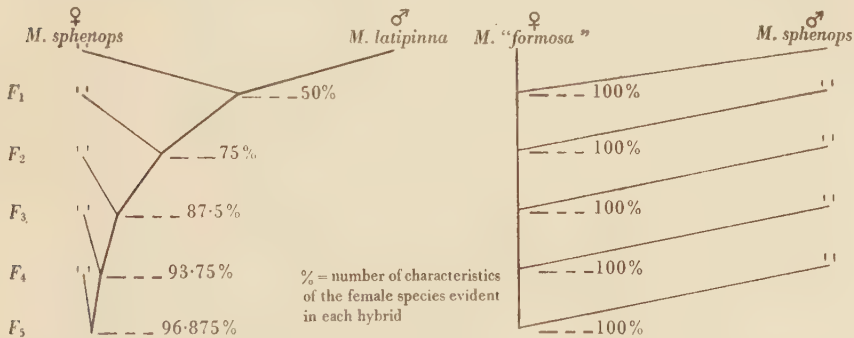
That *M. "formosa"* is a form distinctly different in its reproductive behaviour from *M. sphenops* and *M. latipinna* has been shown by numerous hybridization experiments. When *M. sphenops* and *M. latipinna* are hybridized in the laboratory they produce hybrids of an intermediate character. These F_1 hybrids when back-crossed to females of either *M. sphenops* or *M. latipinna* produce offspring which are intermediate between the F_1 hybrids and the respective parent species. Continued back-crossing produces a gradual reconstruction of the parent species. This indicates that there is a random distribution of the chromosomes in the reduction division of these hybrids. On the other hand, the results of mating successive generations of *M. "formosa"* to males of either *M. sphenops* or *M. latipinna* do not indicate a random distribution of the chromosomes in maturation. All of the offspring produced by such matings have been females which show only the characteristics of the mother form. Text-fig. 12 illustrates the results of five generations of these two types of back-cross hybridization.

In this connexion, mention must be made of the fact that C. L. & L. C. Hubbs have already reared ten generations of *M. "formosa"* in their laboratories and not a single male has appeared. Then, too, it should be

noted that no *M. "formosa"* males have been found, and that in nature this form has undoubtedly continued its existence for many generations by mating with males of *M. sphenops* and *M. latipinna*. These facts indicate that a method of reproduction uncommon among vertebrates is responsible for the continued matroclinous inheritance of the group.

The theory of gynogenesis, advanced by C. L. & L. C. Hubbs, offers one explanation of this type of reproductive behaviour. However, the cytological studies of the present investigation have shown conclusively that *M. "formosa"* has a diploid number of chromosomes. Although this does not disprove the theory of gynogenesis, it does place a second demand upon it, namely the assumption of a duplication of the chromosomes during some stage of development.

An alternative explanation of the presence of a diploid number of chromosomes in this form may be offered, namely that the fertilized



Text-fig. 12. Diagram showing two types of back-cross hybridization.

eggs develop with both maternal and paternal chromosomes. In this case the purely matroclinous inheritance may be explained as follows.

Sperm of *M. sphenops* and *M. latipinna* may fertilize the eggs of *M. "formosa"*. Since *M. "formosa"* is a distinct form, the chromatin introduced by the sperm of these species is foreign to the *M. "formosa"* eggs. Instances showing that foreign paternal chromatin may be non-potent in the presence of maternal chromatin have already been cited. Similarly, the failure of the paternal characteristics to appear in the offspring of *M. "formosa"* seems to be the result of the inability of the chromosomes of *M. sphenops* and *M. latipinna* to affect the nature of the offspring in the presence of the *M. "formosa"* chromosomes. This interpretation offers an explanation of the matroclinous inheritance in *M. "formosa"* for a single generation but does not explain why the succeeding generations exhibit the same phenomenon.

It has been pointed out that repeated back-crossing of *M. sphenops* by *M. latipinna* hybrids to one of the parent species results in a reconstruction of the parent species, while similar back-crossing of *M. "formosa"* produces a pure line of *M. "formosa"*. This indicates that the chromosomal distribution in the maturation of the eggs of *M. "formosa"* may be such that all the paternal chromatin is always eliminated into the polar bodies. This does not seem entirely improbable considered in the light of the findings of Cleland and Schrader. Further, a meiotic behaviour of this type is very significant, for all of the eggs in any and every generation would retain the same type of maternal chromosomes. If a maturation of this type and a non-potency of paternal chromosomes in *M. "formosa"* are admitted, a workable hypothesis for the production of only matroclinous young by each generation of *M. "formosa"* is established. Text-fig. 13 contrasts matroclinous inheritance through gynogenesis and selective maturation.

The idea of a selective association of chromosomes as a solution to problems of ichthyological genetics is not entirely new, since Demerec (1928) suggested that the high number of sex-linked mutants in *Lebistes* might be accounted for by the assumption of association between chromosomes.

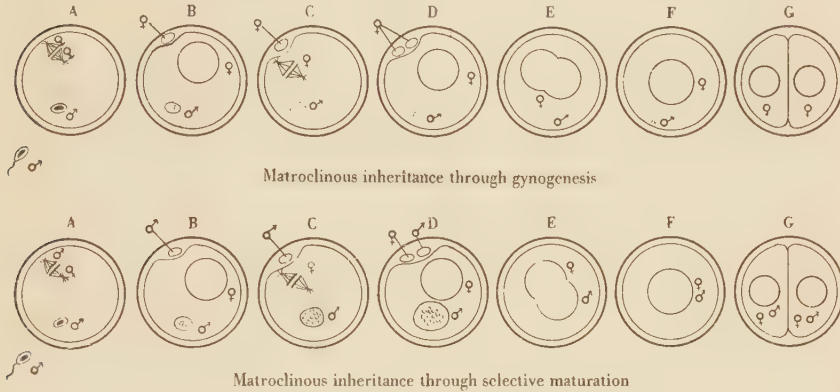
VII. SUMMARY AND CONCLUSIONS

1. When *Mollienisia sphenops* is mated to *M. latipinna* in the laboratory, hybrids of both sexes are produced which mate among themselves. In nature another form, apparently hybrid between the same two species, exists as a purely female population. This form has been called *M. "formosa"*. When these are mated to males of either parental species only female offspring are produced which are exclusively of the maternal type. The aim of this investigation has been to obtain an explanation for the peculiar reproductive condition in this second form of hybrid.

2. *M. "formosa"* females apparently do not produce young by parthenogenesis, for young have never been produced by unmated specimens under observation in the laboratory. Also the presence of males of *M. sphenops* within sight of but out of physical contact with *M. "formosa"* has no apparent effect on the reproductive activity of these females. The males must mate with them if young are to be produced.

3. Dr Carl L. Hubbs suggested that *M. "formosa"* might reproduce by a process of gynogenesis. To test this idea, an attempt was made

to determine when fertilization of the eggs occurs and whether the females develop with a haploid or a diploid number of chromosomes. The results were as follows: (a) It was found that at parturition the ova for the next brood varied greatly in different individuals as to stages of growth and development. Proper stages were not obtained and this



Text-fig. 13. Gynogenesis compared with selective maturation.

Matroclinous inheritance through gynogenesis. The egg is assumed to contain only maternal chromatin. This is illustrated by the spindle showing the maternal chromosomes leaving the equatorial plate in diagram A. Diagrams B, C, and D show the formation of the mature egg with a pronucleus containing only maternal chromatin. However, in gynogenesis the sperm must penetrate the egg if development is to follow. In diagrams A, B, C, D, E, and F the unfit sperm is noticed to disappear. Since *M. "formosa"* develops with a diploid number of chromosomes a nuclear division not followed by a cell division may be assumed. This is illustrated in diagram E. In this way the cleaving egg G develops with only maternal chromatin in its nucleus and the constant pure line of female offspring produced by *M. "formosa"* is explained by a complete absence of paternal chromatin in this form.

Matroclinous inheritance through selective maturation. The egg is assumed to contain both maternal and paternal chromatin. There is, however, a peculiar distribution of the chromatin during meiosis. In diagram A the paternal chromatin is assumed to be arranged on the peripheral side of the spindle. Hence when the first polar body is formed only paternal chromatin is lost, and after the second polar body is formed the mature egg contains only maternal chromatin in its pronucleus. This is illustrated in diagrams B, C, and D. The sperm also forms a pronucleus, figures B, C, and D, which fuses with the egg pronucleus, diagrams E and F. The cleaving egg G thus develops with both maternal and paternal chromatin. In this way the constant pure line of female offspring showing only maternal characters is explained by assuming a non-potency of the paternal sperm in the presence of a normal complement of maternal chromosomes and the foreign cytoplasm of *M. "formosa"*.

made it impossible to study maturation and fertilization stages. (b) A study of spermatogenesis in *M. sphenops*, *M. latipinna*, and the laboratory hybrid between these two species showed that the diploid number of chromosomes is 36. Although no definite counts were obtained for *M. "formosa"*, a comparison of nuclear size in somatic cells of this with nuclear size of similar cells of *M. sphenops* and *M. latipinna* indicated

362 *The Reproductive Behaviour of Mollienisia "formosa"*

that *M. "formosa"* also has a diploid number of chromosomes. This is contingent upon the supposition that nuclear size is, at least in part, dependent on the number of chromosomes.

4. The possibility existed that a differential death-rate of the males and females might be the cause of the purely female populations of *M. "formosa"*. This possibility was tested in various ways as follows: (a) A genetic analysis indicated that if a factor lethal to the males is carried in the Z-chromosome of *M. "formosa"* it would be eliminated in a single generation. (b) Numerous broods of *M. "formosa"* reared by Dr and Mrs Carl L. Hubbs have developed to adult stages without the loss of any individuals. It was also found that the size of the broods was similar in *M. sphenops*, *M. latipinna*, and *M. "formosa"*. This is proof that a differential death-rate does not take place after birth. (c) A thorough study of numerous pregnant specimens of *M. "formosa"* revealed no degenerating embryos which might represent males. This indicates that there is no differential death-rate before birth.

5. A study of sex differentiation in the various species and hybrids of *Mollienisia* showed that there is no tendency toward hermaphroditism in this group and that sex reversal is improbable.

6. In order to throw further light upon the conditions described, and the findings discussed, a study was made of related problems as found in the literature. Some of the more important generalizations are the following: (a) Numerous cases are known in which sex is not irrevocably determined at the time of fertilization. (b) Among fish some groups have heterogametic males, others heterogametic females, while still others exhibit phenotypic sexuality. Sex hormones and external agents may also alter the sex ratio. (c) The successful development of fish hybrids depends upon a favourable reception of the foreign sperm by the egg cytoplasm and upon a harmonious union of the maternal and paternal germ nuclei. (d) Some cases of matroclinous inheritance in hybrid plants seem to be the result of a non-potency of a single set of paternal (foreign) chromosomes in the environment of the egg cytoplasm and the normal complement of maternal chromosomes. (e) In some hybrids, and even in some species, meiosis results in the passage of all the paternal chromosomes to one pole and all of the maternal to the other.

7. From the above study the following conclusions have been reached: (a) *Mollienisia "formosa"*, a natural hybrid of *M. sphenops* and *M. latipinna*, found only as females, acts as a distinct form when back-crossed with the parent species. (b) Existing theories do not offer

a satisfactory explanation for the purely matroclinous inheritance in this form. (c) The results of the present study of *M. "formosa"* and a review of the various theories advanced in connexion with matroclinous inheritance make the following explanation tenable: When eggs of *M. "formosa"* are fertilized by sperm of *M. sphenops* or of *M. latipinna* the resulting embryos contain both maternal and paternal chromosomes. The paternal chromosomes, however, are incapable of affecting the nature of the offspring in the presence of the foreign cytoplasm and maternal chromosomes. When the eggs of the back-cross ripen a selective maturation takes place so that all the paternal chromatin is eliminated into the polar body. In this way each generation retains the maternal form and is in no way affected by the paternal chromatin.

I wish here to express my appreciation for help and encouragement extended by Dr Peter Okkelberg, under whose direction this work was carried out. I am indebted to Dr and Mrs Carl L. Hubbs for supplying the stocks of *Mollienisia*, which made this investigation possible, and for invaluable suggestions and access to unpublished records.

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366 *The Reproductive Behaviour of Mollienisia "formosa"*

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EXPLANATION OF PLATE X

Fig. 1. *Mollienisia "formosa"*. Fig. 2. *Mollienisia sphenops*.

Fig. 3. *Mollienisia latipinna*.

Note. Photographs through the courtesy of Dr Carl L. Hubbs.

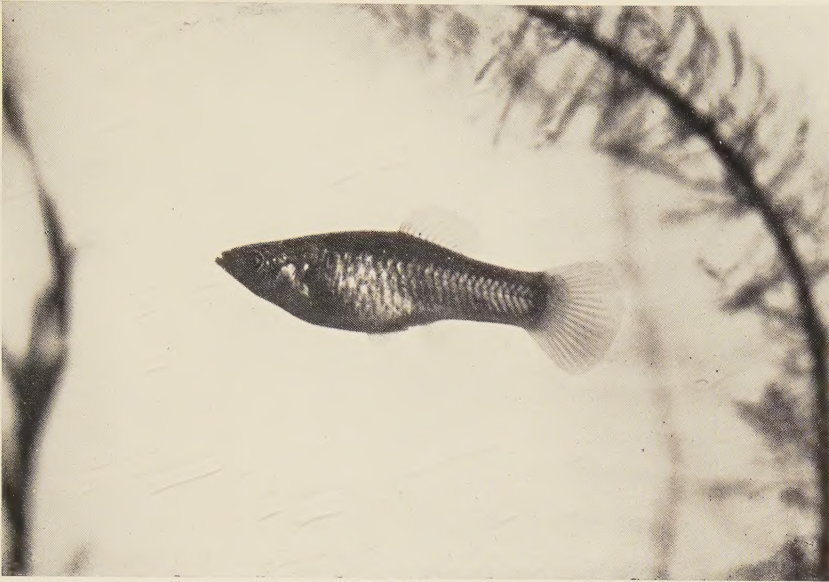


Fig. 1.



Fig. 2.



Fig. 3.

